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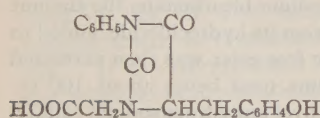
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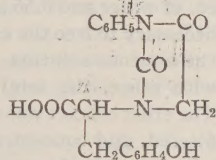
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Hydantoins. LII.¹ The Synthesis of N-3-Phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic Acid from Tyrosine-N-acetic Acid²BY ELINOR WARE³

The purpose of this investigation was to study the effect of substituent groups on the cyclization of a derivative of hydantoic acid which might theoretically undergo ring closure to give either of two isomeric hydantoins. A large number of α -amino acids and the symmetrical imino dibasic acid, iminodiacetic acid, have been converted into hydantoic acid and hydantoin derivatives through the action of aryl isocyanates and isothiocyanates,⁴ but similar reactions of an unsymmetrical imino dibasic acid of the type of tyrosine-N-acetic acid have not been studied. Reactions of isocyanates with unsymmetrical imino dibasic acids are of especial interest since, for example, the substituted hydantoic acid prepared from tyrosine-N-acetic acid by treatment with phenyl isocyanate might be expected to undergo cyclization to give either of two isomeric hydantoins: N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), or N-3-phenylhydantoin-N-1-*p*-hydroxybenzylacetic acid (II).



I



II

The fact that only one of these two isomeric hydantoins was isolated when this particular reaction was studied would appear to indicate that one of these hydantoins, because of the relative positions of its substituent groups, is formed much more readily from the corresponding acyclic hydantoic acid derivative than is its isomer.

When tyrosine-N-acetic acid was allowed to react with phenyl isocyanate in alkaline solution,

(1) Previous papers of this series: L, Herbst and Johnson, *THIS JOURNAL*, **54**, 2463 (1932); LI, Haring and Johnson, *ibid.*, **55**, 395 (1933).

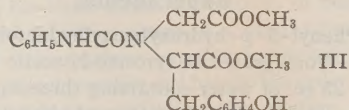
(2) Constructed from a dissertation presented in June, 1937, by the author to the Faculty of the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.—T. B. Johnson.

(3) University Scholar in Chemistry, Yale Graduate School, 1936-37.

(4) Marckwald, Neumark and Stelzner, *Ber.*, **24**, 3278 (1891); Mouneyrat, *ibid.*, **33**, 2399 (1900); Neuberg and Manasse, *ibid.*, **38**, 2359 (1905); Bailey and Randolph, *ibid.*, **41**, 2505 (1908); Brautlecht, *J. Biol. Chem.*, **10**, 139 (1911); Bailey and Snyder, *THIS JOURNAL*, **37**, 935 (1915).

the product obtained on neutralizing the cold alkaline reaction mixture was not the phenylureido derivative of tyrosine-N-acetic acid, but the closed hydantoin, N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I). This result is quite in accordance with the work of other investigators, who found that certain substituted hydantoic acids were so unstable that they underwent spontaneous ring closure in cold neutral solutions.⁵ An attempt to prepare the acyclic phenylureido derivative of tyrosine-N-acetic acid by decomposing its copper salt with hydrogen sulfide gave similar results, the closed hydantoin (I) again being obtained when the filtrate from the copper sulfide was concentrated under reduced pressure at room temperature.

While the free acyclic dibasic phenylureidic acid was not isolated because of its strong tendency to undergo ring closure, it was obtained in the form of its dimethyl ester (III).



This ester was obtained by treating the dimethyl ester of tyrosine-N-acetic acid with phenyl isocyanate in ether solution. Since this substance is only slightly soluble in ether, it was precipitated in crystalline form from the reaction mixture. This dimethyl ester of the phenylureido acid could be made to undergo cyclization to a hydantoin derivative by either of two methods. When boiled for half an hour with 25% hydrochloric acid, it was completely converted into N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), the same compound which had been obtained when the alkaline reaction mixture of tyrosine-N-acetic acid and phenyl isocyanate was neutralized with mineral acid. Boiling of this ester in neutral aqueous solution for somewhat longer periods of time brought about the formation of the methyl ester of this same hydantoin-N-1-acetic acid.

The hydantoin prepared from tyrosine-N-acetic acid by the methods outlined above was proved

(5) Friedman, *Beitr. Chem. physiol. Path.*, **11**, 160 (1908); Brautlecht, *J. Biol. Chem.*, **10**, 139 (1911); Hahn and Endicott, *THIS JOURNAL*, **60**, 1040 (1938).

through synthesis to be N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I). N-3-Phenyl 5-anisalhydantoin was prepared according to the method of Wheeler and Hoffman,⁶ and then converted into ethyl N-3-phenyl-5-anisalhydantoin-N-1-acetate by allowing ethyl chloroacetate to react with its sodium derivative in alcohol solution. By treatment with hydriodic acid in the presence of red phosphorus, this unsaturated ester was transformed into N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), which proved to be identical with the substance obtained through the action of phenyl isocyanate on tyrosine-N-acetic acid. Therefore, in these particular experiments only one of the two theoretically possible isomeric hydantoins was obtained through the methods of ring closure employed.

This N-3-phenylhydantoin (I) will break down under alkaline hydrolysis to form tyrosine-N-acetic acid, carbon dioxide, and aniline. This decomposition follows the same course as that of the corresponding N-3-methyl derivative, which was found by Hahn and Renfrew to be converted by intensive alkaline hydrolysis into tyrosine-N-acetic acid, carbon dioxide, and methylamine.⁷

Experimental

N-3-Phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic Acid (I).—Four grams of tyrosine-N-acetic acid was dissolved in 25 cc. of water containing three equivalents (2.8 g.) of potassium hydroxide, 2.0 cc. of phenyl isocyanate (a small excess) slowly added to the cold solution during a period of fifteen minutes, and the mixture mechanically stirred for seven hours while cooled in an ice-bath. The resulting milky solution was allowed to stand overnight at room temperature, a little diphenylurea was removed, and the alkaline filtrate was again cooled and neutralized with an amount of hydrochloric acid equivalent to the potassium hydroxide used. After all of the hydrochloric acid had been added, a gum precipitated and then slowly crystallized. This precipitate weighed 4.8 g. and melted at 198–201° to a clear oil. After recrystallization from 200 cc. of water, the melting point was raised to 202–203°. Additional quantities of this substance were obtained by concentration of the mother liquor, so that the yield was practically theoretical. This substance was shown to be N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid through comparison with a sample of this substance prepared by another method, with which it proved to be identical.

Anal. Calcd. for $C_{18}H_{16}O_6N_2$: N, 8.24. Found: N, 8.25, 8.28.

This substance may be recrystallized most satisfactorily from boiling water, and can be made to separate in fairly large colorless crystals when its hot aqueous solution is

cooled very slowly. It is quite soluble in alcohol and insoluble in ether.

This same hydantoin (I) was obtained by the decomposition of the copper salt of phenylureido-tyrosine-N-acetic acid with hydrogen sulfide. The copper salt was prepared by adding the theoretical amount of cupric chloride to an aqueous solution of the dipotassium salt of the phenylureido acid. Since it was almost insoluble in both alcohol and water, it was not recrystallized or analyzed. This blue copper salt was suspended in 95% alcohol, and gaseous hydrogen sulfide led into the mixture. After the removal of the precipitated copper sulfide, the filtrate was concentrated under reduced pressure at room temperature. When practically all the alcohol had been removed, the residue crystallized to give the same hydantoin (I) which previously had been obtained from solutions of the dipotassium salt of the phenylureido acid.

This hydantoin (I) was also prepared from the dimethyl ester of phenylureido-tyrosine-N-acetic acid (III) by boiling the latter with 25% hydrochloric acid. Two-tenths of a gram of the phenylureido ester was boiled for thirty minutes with 50 cc. of 25% hydrochloric acid. When the solution was cooled, a precipitate melting at 195–200° was obtained. After recrystallization from water it melted at 202–203°.

$CH_3OOCCH_2NHCH(CH_2C_6H_4OH)COOCH_3$, Dimethyl Tyrosine-N-acetate.—The hydrochloride of dimethyl tyrosine-N-acetate was prepared according to the method used by Hahn and Litzinger, through the treatment of tyrosine-N-acetic acid with a solution of dry hydrogen chloride in methyl alcohol.⁸ In order to obtain the free ester, 2.0 g. of the ester hydrochloride was dissolved in 15 cc. of water and 0.55 g. of sodium bicarbonate, the amount necessary to free the ester from its hydrochloride, added to the aqueous solution. The free ester was then extracted with ether, the total volume used being about 100 cc. The ether extract was dried over anhydrous sodium sulfate, filtered, and concentrated to a small volume. When the ether was nearly evaporated and the free ester began to crystallize, water was added carefully in small portions to complete the crystallization of the ester. Dimethyl tyrosine-N-acetate is very soluble in both methyl alcohol and ether, but only slightly soluble in water. It was recrystallized by dissolving in a small amount of methyl alcohol and precipitating with water. It melts to a clear oil at 84–85°.

Anal. Calcd. for $C_{18}H_{17}O_6N$: N, 5.24. Found: N, 5.25, 5.02, 5.08.

Dimethyl Phenylureido-tyrosine-N-acetate (III).—The dimethyl ester of tyrosine-N-acetic acid was converted quantitatively into its phenylureido derivative by treatment with phenyl isocyanate in ether solution. One gram of dimethyl tyrosine-N-acetate was dissolved in 100 cc. of ether, and 0.8 cc. of phenyl isocyanate slowly added. The mixture was stirred in an ice-bath for three hours and then filtered from the precipitate which had begun to form at the end of twenty minutes. This precipitate consisted of the phenylureido derivative mixed with a small amount of diphenyl urea. It was purified by dissolving it in a small amount of methyl alcohol, filtering out the diphenyl urea, concentrating the alcoholic solution somewhat under

(6) Wheeler and Hoffman, *Am. Chem. J.*, **45**, 375 (1911).

(7) Hahn and Renfrew, *This Journal*, **47**, 147 (1925).

(8) Hahn and Litzinger, *ibid.*, **54**, 4664 (1932).

reduced pressure, and reprecipitating the phenylureido ester by the addition of ether. The melting point was 124–125° with evolution of a gas. This ester is very soluble in methyl alcohol, only slightly soluble in ether, and insoluble in water. The analyses of recently recrystallized samples indicate the presence of a molecule of methyl alcohol of crystallization.

Anal. Calcd. for $C_{20}H_{22}O_6N_2 \cdot CH_3OH$: N, 6.70. Found: N, 6.66, 6.79, 6.77.

Since heating will cause partial decomposition of this ester, it cannot be freed from solvents by this method. The methyl alcohol appears to be lost after long standing over dehydrating agents, as indicated by the analyses of a sample which had stood in a desiccator over calcium chloride for several months, although the melting point was unchanged.

Anal. Calcd. for $C_{20}H_{22}O_6N_2$: N, 7.26. Found: N, 7.33, 7.36.

$CH_3OOCCH_2NCON(C_6H_5)COCHCH_2C_6H_4OH$, Methyl N-3-Phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetate.—This ester could be prepared by either of two methods: (a) the esterification of the corresponding acid (I) with methyl alcoholic hydrogen chloride, or (b) the boiling of dimethyl phenylureido-tyrosine-N-acetate (III) in aqueous solution.

(a) Five-tenths of a gram of N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I) was dissolved in 25 cc. of methyl alcohol, and the solution saturated with dry hydrogen chloride. The reaction mixture was then heated for several hours and finally evaporated to an oil. Treatment of this oil with methyl alcohol-water mixtures brought about the crystallization of the ester, m. p. 140–141°. It was recrystallized by dissolving in a few cc. of methyl alcohol and reprecipitating through the addition of water.

Anal. Calcd. for $C_{19}H_{18}O_6N_2 \cdot CH_3OH$: N, 7.25. Found: N, 7.19, 7.32, 7.19.

(b) The preparation of this ester by boiling the dimethyl ester of phenylureido-tyrosine-N-acetic acid (III) in neutral aqueous solution was not so satisfactory, since only partial conversion occurred even after several hours of heating. One gram of the phenylureido ester was boiled with 200 cc. of water for one hour, during which time a large amount of gummy precipitate was formed. By repeated extraction of this gum with boiling water, several precipitates of the methyl ester of the hydantoin-N-1-acetic acid (I) were obtained. The rest of the gum was converted into the corresponding acid (I) by boiling it with 25% hydrochloric acid.

$C_2H_5OOCCH_2NCON(C_6H_5)COC=CHC_6H_4OCH_3$, Ethyl N-3-Phenyl-5-anisalhydantoin-N-1-acetate.—In order to prove that the substance obtained through the action of phenyl isocyanate on tyrosine-N-acetic acid was N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), it was necessary to synthesize this compound by another method which could leave no doubt of the configuration of the compound. The first step in this synthesis was the preparation of N-3-phenyl-5-anisalhydantoin,

$NHCON(C_6H_5)COC=CHC_6H_4OCH_3$, which was then con-

verted into ethyl N-3-phenyl-5-anisalhydantoin-N-1-acetate. Five grams of N-3-phenyl-5-anisalhydantoin, prepared by the method of Wheeler and Hoffman⁹ from N-3-phenylhydantoin,^{9,10} was added to a solution of 0.43 g. of sodium in 50 cc. of absolute alcohol. The mixture was then heated for fifteen minutes, the phenylhydantoin dissolving as soon as the solution was hot, and 2.3 g. of ethyl chloroacetate (1.1 equivalents) added, together with 10 cc. more of absolute alcohol. The solution was now heated under reflux for three and a half hours. A heavy precipitate of sodium chloride began to separate at the end of one hour. At the termination of the heating period, the reaction mixture was poured into an equal volume of water, when a heavy precipitate was formed. This precipitate was extracted with chloroform in order to separate the reaction product from any unchanged N-3-phenyl-5-anisalhydantoin, and a residue of 2.5 g. of this hydantoin was obtained. The chloroform extract was evaporated almost to an oil and then treated with alcohol, when crystals of the reaction product, ethyl N-3-phenyl-5-anisalhydantoin-N-1-acetate, were obtained. After recrystallization from absolute alcohol, they melted at 89–91°.

Anal. Calcd. for $C_{21}H_{20}O_6N_2$: N, 7.38. Found: N, 7.22, 7.32.

This ester was converted by treatment with hydrogen iodide and red phosphorus into N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), reduction of the double bond, demethylation, and hydrolysis of the ester group taking place under the action of this reagent. Five-tenths of a gram of the unsaturated hydantoin ester was refluxed for three hours with 10 cc. of concentrated hydriodic acid in the presence of a small amount of red phosphorus. The mixture was then subjected to distillation under reduced pressure at 110–120° in order to remove the methyl iodide formed during the reaction, as well as the excess of hydrogen iodide. Small amounts of water were added from time to time, and the distillation continued until the distillate gave no test for iodide ions. The residue was dissolved in hot water, filtered from red phosphorus, and the solution concentrated to five cc. Crystals of N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I), melting at 202–203° after recrystallization from hot water, were obtained from the cool solution. This hydantoin proved to be identical with the substance prepared from tyrosine-N-acetic acid and phenyl isocyanate, as shown by a mixed melting point determination, as well as by a comparison of their other properties.

Hydrolysis of N-3-Phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic Acid with Barium Hydroxide.—Hot aqueous solutions of 1 g. of N-3-phenyl-5-*p*-hydroxybenzylhydantoin-N-1-acetic acid (I) and 3 g. of barium hydroxide were mixed and heated on a steam-bath for five hours, the total volume being kept between 10 and 15 cc. During the hydrolysis the odor of aniline could be detected and a white precipitate was formed. The hot mixture was filtered and the filtrate concentrated to a small volume, when a second precipitate was obtained by the addition of alcohol. The two precipitates were suspended in hot water, and the barium exactly neutralized with sulfuric

(9) Paal, *Ber.*, **27**, 975 (1894).

(10) Mouneyrat *ibid.* **33**, 2399 (1900).

acid, the barium sulfate being subsequently filtered from the hot solution. After concentration of the filtrate, a precipitate of the imino dibasic acid, tyrosine-N-acetic acid, was obtained. This substance was identified by conversion into its characteristic hydrochloride, m. p. 212–213° with evolution of a gas, by treatment with 1:1 hydrochloric acid.⁸

Summary

Tyrosine-N-acetic acid, an unsymmetrical imino dibasic acid, will react with phenyl isocyanate to

form a phenylureido dibasic acid which appears to be stable only in the form of its salts or esters. The free phenylureido acid undergoes spontaneous ring closure in cold aqueous solutions to give a trisubstituted hydantoin. Although the phenylureido derivative of tyrosine-N-acetic acid may theoretically undergo cyclization to give either of two isomeric hydantoins, only one of these isomers was obtained under the conditions studied.

NEW HAVEN, CONN.

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